REMARKS/ARGUMENTS

Claims 13-14 and 38-49 are currently pending. Claims 13, 42, 43 and 49 are amended herein. Clerical errors are corrected in claims 13, 42 and 49 no new matter is added by way of these amendments. Specific support for these amendments are found in the as-filed specification at, for example, paragraphs 237 and 352 and Example F, beginning at paragraph [0197]. No new matter is added by way of these amendments. This application now stands in allowable form and reconsideration and allowance is respectfully requested.

Double Patenting Rejection

Claims 13-14 and 38-49 are provisionally rejected on the grounds of nonstatutory obviousness-type double patenting as being unpatentable over claims 1, 12-14, and 38-49 of copending Application No. 10/781,499.

This rejection is overcome, at least, for the following reasons.

A terminal disclaimer over claims 1, 12-14 and 38-49 of copending Application No. 10/781,499 is provided herewith. Withdrawal of the rejection is therefore respectfully requested.

Claim Objections

Claim 49 is objected to because of the following informalities: Claim 49 is directed to the method of claim 13, wherein the microorganism is *E. coli* and *C. glutamicum.*

This objection is now moot because the claim has been amended herein to recite *E. coli* **or** *C. glutamicum*. No new matter is added by way of this amendment. Applicants appreciate the Examiner's careful review of the specification and suggestions regarding the claim language.

Claim Rejections – 35 USC §112

Claim 42 is rejected under 35 USC § 112, first paragraph, as failing to comply with the written description requirement.

This rejection is overcome, at least, for the following reasons.

The claim has been amended to read that the cosubstrate is added to the media in step (b) as described in the specification in Example F and correcting for a clerical error. No new matter is added by way of the amendment. Applicants appreciate the Examiner's careful review of the specification.

Claim Rejections – 35 USC §103

Claims 13-14, 38-41 and 43-49 are rejected under 35 USC § 103(a) as being unpatentable over Richaud et al. (J. Biological Chemistry, December 25, 1993;268(36):268(27-35) in view of Short (U.S. Patent Publication No. 2005/0124010).

This rejection is overcome, at least, for the following reasons.

First, with respect to the Office's comment that the terms "compensatory metabolic pathway" and "directed genetic modification" are not explicitly defined by the specification, Applicants respectfully disagree. However, in the interest of furthering prosecution the term "compensatory" has been omitted. Applicants note that the evolution of the metabolic pathway is described throughout the specification at, for example, [0064]-[0066] and throughout the Examples. Specifically, the "the metabolic pathways to be evolved are generally selected from among the synthetic pathways of amino acids . . . nucleic acids, . . . lipids or . . . sugars." [0064]. The "evolution of the biosynthesis pathway" [0205], and the "new metabolic pathway" [0236] are further described by the in filed specification U.S. published application 2005/0054040A1.,

Further, the process of "directed genetic modification" is well understood by those of skill in the art and is described in the specification, for example:

"Those skilled in the art know the protocols used to modify the genetic characters of microorganisms.

The inactivation of a gene is carried out preferably by homologous recombination. (Datsenko, K. A.; Wanner, B. L. (2000), One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products. Proc. Natl. Acad. Sci. USA 97: 6640-6645). The principal of a protocol is briefly as follows: a linear fragment obtained in vitro is introduced into the cell; this fragment comprises the two regions flanking the gene and at least one gene of selection between these two regions (generally a gene of resistance to an antibiotic); the fragment

therefore presents an inactivated gene. The cells that have undergone a recombination event and have integrated the fragment are then selected by spreading them on a selective medium. The cells that have undergone a double recombination event in which the native gene has been replaced by the inactivated gene are then selected. This protocol can be improved by using positive or negative selection systems to increase the rate of detection of double recombination events." Pat Pub. US 2005/0054060 at [0054]-[0055] (published version of the as filed 10/546,139).

Further, the process of such directed genomic modification is discussed in the specification at, for example, paragraphs [0070]-[0094] and [0114]-[0120].

The Office Mischaracterizes Richaud

Richaud is cited for the proposition that it teaches all the elements of instant claim 13 with the exception of step (d) "isolation of the evolved protein." Applicants respectfully disagree. Specifically, claim 13 requires the "directed genetic modification of a microorganism, "wherein the production or consumption of a substrate is inhibited when the modified microorganism is grown on a defined medium, wherein the ability of the modified microorganism to grow is impaired." In contrast, Richaud teaches the directed genetic modification of a microorganism and, in particular, the joint overexpression of the *metB* gene and disruption of *metC* gene. Further, Richaud does not attempt to "evolve" any metabolic pathways. On the contrary, the purpose of Richaud is "to install a biosynthetic pathway for the thioether analog of meso-diaminopimelate, meso-lanthionine (Fig. 1), in the metabolism of *E. coli* through the smallest possible number of genetic changes." Richaud at 26828. As discussed, ***Richaud, teaches replacing the substrate used in thioether biosynthesis to generate different sulfur-containing amino acids (meso-lanthionine and L-allo-cystathionine) used in cross-linking the bacterial cell wall.*** (See, for example, Abstract). In effect, Richaud teaches engineering the bacteria to use different substrates to result in different products incorporated in the cell wall peptidoglycan "in the smallest number of genetic changes". In contrast, the instant claims require, modifying a gene to inhibit growth on a substrate and evolving a compensatory metabolic pathway to arrive at the ***same end-product***. Thus, Applicants respectfully submit that while Richaud does not teach step (d) of independent claim 13, Richaud, in fact, does not teach

steps (a) through (c) as required by claim 13 either. Therefore, for this reason alone, the rejection is overcome and should be withdrawn. Applicants respectfully request same.

Further, Richaud et al. add that "*Only the disruption of two genes and the overexpression of another were required*" and that "*this commitment did not result from natural selection but was rationally set up in their genome*" (p. 26834, col. 2, 2nd para.)(emphasis added). Applicants respectfully submit that if no alteration of enzyme specificities occurred, *if no natural selection occurred, than neither could the evolution of a metabolic pathway occur*. Therefore, no evolved microorganism was produced, as is required by the claim. This is succinctly stated by Richaud, "*[T]he genetic changes that we enforced in our strains for remodelling their chemical constitution implied no alteration of enzyme specificities or catalytic activities.*" Id. Therefore, for this reason alone, the rejection is overcome and should be withdrawn. Applicants respectfully request same.

In addition, for the Examiner's convenience, Applicants have prepared a chart illustrating the difference between Richaud and the instantly claimed invention.

	The present invention	**Richaud et al.**
Step a	Directed genetic modification of a microorganism, suppressing an enzymatic activity, wherein growth is impaired (see paragraphs 74, 75, 76, 77).	Directed genetic modification of a microorganism, in particular overexpression of the metB gene and disruption of metC gene
Step b	Genetically modified microorganism are cultivated for numerous cycles in a flask, in a medium comprising methylmercaptan or sodium methylmercaptide, or sulphur compound as co-substrate (see [75]) and free of the metabolite, the production of which is inhibited by the previously done modification [21]. This is a step of evolution such as defined in the specification: see in particular paragraphs [3], [78], [199], [204], [208].	No corresponding step See also the materials and methods "Growth of bacterial strains", stating that bacteria are **routinely** grown.
Step c	Selection of microorganisms able to grow on said defined medium,	Selection of microorganisms able to grow on lanthionine and lysine (see page 26829,

	wherein at least one protein has evolved.	column 1), but without any evolution of protein : *"this commitment did not result from natural selection but was rationally set up in their genome"* (p. 26834, column 2, second paragraph).
Step d	Isolation of the evolved protein	No evolved protein

Short Does Not Remedy The Defects Of Richaud

Short is cited for the proposition that it teaches isolating the evolved protein. Applicants respectfully submit that the Office has mischaracterized Short. In addition, Short not remedying the defects of Richaud cannot make the instant invention obvious. First, Applicants note that Short is not prior art to the instant application having a publication date of June 9, 2005, well after the February 17, 2004 priority date of the instant application. However, Short, in any case, does not rectify the deficiencies of Richaud. Specifically, the Office cites to paragraphs [1062]-[1063] of Short for the proposition that it teaches isolating cells which produce a desired metabolite. Applicants respectfully disagree. The passage cited by the Office is taken out of context. Short does not teach producing an "evolved cell" but rather teaches "The method can further comprises selecting a cell comprising a newly engineered phenotype. The selected cell can be isolated. The method can further comprise culturing the selected or isolated cell thereby generating a new cell strain or cell line comprising a newly engineered phenotype. The method can further comprise isolating a cell comprising a newly engineered phenotype." [1062]. Thus, it is clear that the cell selected by Short is not grown under any selective pressure but comprises a modified genome having an *engineered* phenotype. The cell therefore cultured has the same engineered phenotype as the parent. Therefore, applicants respectfully submit that Short, as with Richaud, does not teach limitation of (a)-(c) required by independent claim 13. The rejection over Richaud in view of Short being thus overcome, withdrawal is respectfully requested.

Short Does Not Teach The Limitation Of Isolating The Evolved Protein

The Office refers to paragraph [1070] of Short for the proposition that Short teaches isolating the protein of step (d). Applicants respectfully submit that the Office has misrepresented Short. Specifically, the passage cited by the Office states "any metabolic parameter can be **measured**." [1070] (emphasis added). In this context, Short notes that "In

practicing the methods of the invention, any metabolic parameter can be measured. In one aspect, several different metabolic parameters are evaluated in the cell culture. The metabolic parameters can be measured at the same time or sequentially. One exemplary metabolic parameter is rate of cell growth, . . . by, e.g., optical density of the cell culture. Another metabolic parameter measured comprises a change in the expression of a polypeptide. Changes in the expression of the polypeptide can be measure by any method, e.g., a one-dimensional gel electrophoresis, a two-dimensional gel electrophoresis, a tandem mass spectography, an RIA and ELISA an immunoprecipitation and a Western blot." Id. Each of these techniques does allow **quantification**, or **measurement** of a specific polypeptide, however, **none of them result in isolation of that polypeptide**.

Specifically, Applicants submit that a one-dimensional gel electrophoresis, assuming no other proteins of similar molecular weight are present in the culture, results and the peptide embeded in a gel. Without further manipulation, the peptide has not been isolated, but in the presence of the appropriate standards it can be measure or quantified as taught by Short. Two-D gel electrophoresis has the same limitations as one-D with the exception that the second dimension allows further separation of the peptide *within* the gel based on charge. Tandem mass spectography allows for measurement of a molecule but also results in its destruction due to bombardment. RIA, ELISA, immunoprecipitation and Western blot are all methods of measurement, e.g., quantitation, but do not allow for isolation, as in each case, the peptide of interest is complexed to an antibody, embedded in a gel, run on a dipstick, etc. Isolation of a protein from an antibody/antigen complex requires much further manipulation to retrieve the antigen from the antibody complex e.g., various salt washes. Therefore, it is clear that Short does not teach or even contemplate the *isolation* of the discussed polypeptide because none of the methods provided actually result in the isolation of the peptide without considerable further manipulation. In fact, some, such as mass spectroscopy, are purely methods of measurement and could never result in the isolation of any molecule because the process of measurement itself results in the destruction of the molecule. Therefore, Applicants respectfully submit that, in fact, Short teaches methods of "measurement" not isolation as evidenced by the plain meaning of the word "measured" used by Short. Therefore, for this reason alone, the rejection is overcome and should be withdrawn. Applicants respectfully request same.

Neither Richaud Nor Short Alone Or In Combination Teach The Limitations Of The Instant Claimed Invention

Independent claim 13 requires generating a directed genetic modification in a microorganism to yield a modified microorganism such that its growth on a substrate is inhibited. Neither Richaud nor Short teach such modification. Specifically, Richaud is directed towards introducing an entirely new synthetic pathway via the introduction of as few genes as possible to result in the incorporation of non-native sulfur-containing molecules within the peptidoglycan of a cell wall. Richaud does not teach or even contemplate the use of non-native substrates to result in a native product and, in fact, Richaud teaches "the genetic changes that we enforced in or strains for remodeling their chemical constitution implied no alteration of enzyme specificities or catalytic activities." 26834. Thus, Richaud teaches away from the present invention because Richaud teaches enforcing a new chemical constitution on the microbe, (See, Richaud at 16834) not allowing the microbe to evolve a compensatory metabolic pathway, as is required by the claims.

Short further teaches away from the invention. Short is directed to "cellular and whole organism engineering." Abstract, [0001]. As taught by Short, "The instant invention solves these and other problems by providing a method of producing genetically altered organisms having a large number of stacked traits that are differentially activatable. Upon purchasing such a genetically altered organism, the purchasing customer has the option of selecting an paying for particular traits amount the total that can then be activated differentially." Short at [0010]. Applicants respectfully submit that the goal of "purchasing" an organism with a plurality of "stacked traits" is diametrically opposed to evolving a microorganism and isolating an evolved protein. One of skill in the art reading Short's description of cellular transformation and "producing a genetically altered organism having a large number of stacked traits" would not consider "generating a directed modification in a gene of interest" in order to impair the growth of the microorganism in order to evolve the microorganism. The instant invention and Short are simply incompatible.

The Combination Of Richaud And Short Would Not Result In The Instant Invention

Further, Applicants submit that if one of skill in the art did combine Richaud with Short, they would not arrive at the instant invention. Specifically, Richaud teaches "installing" whole new "biosynthetic pathways . . . through the smallest possible number of genetic changes." Richaud at 26828. The method includes, apparently, whatever genetic manipulations are necessary. The method including simultaneous gene deletions, overexpressions, mutations etc. Thus, Applicants respectfully submit that the combination of Richaud and Short merely provides a microorganism with a profundity of whatever "stacked" traits can be engineered into the microorganism. The only apparent limitation being the size of the traits or genes being desired to integrate into the genome or to include on a plasmid. Whatever result, it would not be a microorganism that resulted from a directed modification in a gene of interest and resulted in the inhibition of growth as is required, in part, by claim 13. Therefore, for this reason alone, the rejection is overcome and should be withdrawn. Applicants respectfully request same.

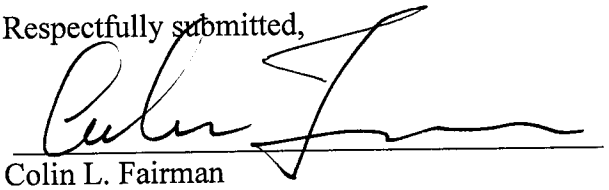
CONCLUSION

In view of the foregoing, it is respectfully submitted that each of the pending claims is in condition for allowance, and a Notice of Allowance is earnestly solicited. The Examiner is invited to contact the undersigned attorney at (612) 321-2237 with any questions, comments or suggestions relating to the referenced patent application.

This response is being submitted on or before March 24, 2009 along with a fee of $65.00 making this a timely response. It is believed that no additional fees are due in connection with this filing. However, the Commissioner is authorized to charge any additional fees, including extension fees or other relief which may be required, or credit any overpayment and notify us of reason, the Commissioner is hereby authorized to deduct said fee from Fulbright & Jaworski L.L.P. Account No.: 50-1212/CABR (34076/US/2).

CUSTOMER NUMBER **38824**

Respectfully submitted,



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